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Dr. Robert J. Byrne
University of Maryland

Studies: Studies on Arbovirus Infections in Equines.

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A series of experim-nts ries of experiments were conducted in which burros were subjected to multiple exposures to G oup Aexposures to Croup A arboviruses. Production of broadly reactive CF antibodies was found to depes was found to depend on the sequence of different viruses injected. The highest broad spectrum CFst broad spectrum CF reactions appeared in EEE immune burros after challenge with virulent VEE v with virulent VEE virus. Primary inoculation with EEE, WEE, and VEE gave good specific CF and HAI specific CF and HAI antibody response. In WEE immune burros, challenged with Sindbis virus, antibody bis virus, antibody response was insignificant.

The immune response of dimmune response of domestic chickens of various ages to inoculation with EEE virus is in progress. Bl is in progress. Blood virus levels in younger birds were of a higher order and persist longer than persist longer than in older birds inoculated with the same quantity of virus.

Guaroa virus was cultivaoa virus was cultivated on 6 different tissue culture lines. The virus is being characterized and thcharacterized and the immunologic response is being measured in mice, rabbits, and chickens. Unsucand chickens. Unsuccessful attempts were made to produce a hemagglutinin to Cache Valley-like virus revalley-like virus recently isolated.

Of 156 non-vaccinated po56 non-vaccinated ponies, neutralizing antibodies to EEE were detected in 40 with HA antibodies to Eh HA antibodies to EEE detectable in 19.

A serologic survey indicrologic survey indicates that a Cache Valley-like virus is rather widely disseminated in domestseminated in domestic animals in the tidewater areas of Maryland and Virginia.

I. Epidemiological Studies

Operations at the Assateague Research Laboratory have continued on Wallop's Island, Virginia, as a cooperative effort among the Division of Communicable Diseases and Immunology, Walter Reed Army Institute of Research (WRAIR), Fish and Wildlife Service, U. S. Department of Interior, and the Department of Veterinary Science, University of Maryland. From this base, the studies reported below have been conducted.

A. Eastern equine encephalomyelitis (EEE) virus.

1. No EEE virus was isolated from more than 9000 mosquitoes collected by the WRAIR group in the Chincoteague-Assateague Island complex during 1962. Only 19 of 1327 bird plasmas were found to have EEE neutralizing antibodies.

2. Tests were completed on serum samples obtained in 1961-1962 from herds of non-vaccinated ponies in the eastern counties of Virginia which lie between the Chesapeake Bay and the Atlantic Ocean. Serum samples were collected by the U. of M. group from 156 ponies in 3 different locations and tested by serum dilution neutralization (NT) and hemagglutination-inhibition (HAI) tests. Results are shown in the table.

Serologic Tests for Antibodies to EEE in Ponies

Location	# of Sera Tested	# of Sera Positive at 1:10 or >	
		NT (10^2-10^3 LD_{50} of virus)	HAI (4 units of antigen)
Chincoteague, Va.	91	25	10
Assateague, Va.	16	11	8
Pungoteague, Va.	49	4	1

All sera that were positive by the HAI test had an NT titer $\geq$ 1:80. It is evident from these results and those obtained in the past that there is a higher frequency of exposure to EEE in the Chincoteague-Assateague area than in an area a short distance inland.

3. There were no laboratory confirmed cases of EEE in Maryland horses and ponies in 1962, although specimens were received from 13 suspect horses.

B. Cache Valley-Like Viruses.

Isolation of a Cache Valley-like virus from mosquitoes had been accomplished in 1961 by the WRAIR group. It was determined that the "Anopheles barbirostris-crucians complex" of mosquitoes was apparently frequently involved in the dissemination of the Cache Valley-like virus. A serological survey for neutralizing antibodies to Cache Valley virus was conducted by the WRAIR group on serum samples collected by the U. of M. group from domestic animals in the Tidewater, Piedmont, and mountainous areas of Maryland and Virginia indicates a high rate of infection in horses and cattle. In the Tidewater area, 114 of 157 animals had neutralizing antibodies to Cache Valley virus, 28 of 75 in the Piedmont area, and 11 of 51 in the Appalachian area. It would appear from these preliminary tests that this agent has been rather widely disseminated among domestic animals in Tidewater Maryland and Virginia for several years. Further investigations of the ecology of this virus are in progress.

II. Experimental Studies.

A. Group A arboviruses in equines

CF Results on Serum Samples from Burros Subjected to Sequential Exposure to Group A Arboviruses*

Antigen and Units		Serum GF 137 3/7/61	GF 140 3/7/61	GF 144 11/17/61	GF 308 4/10/62	GF 325 3/19/63	GF 325 4/9/63	GF 331 3/19/63	GF 331 4/9/63
EEE	16	128+**	128+	32	64	0	128+	0	64
	8	128+	128+	16	128	0	128+	0	64
WEE	16	16	32	128	128+	0	128	0	32
	8	16	16	128+	128+	0	64	0	32
VEE	16	128+	128+	128+	16	0	128+	0	64
	8	128+	128+	128+	16	0	128+	0	64
AMM 2021	16	32	32	32	32	0	128	0	16
	8	64	64	64	32	0	128	0	16
AMM 2354	16	64	64	64	32	0	128	0	64
	8	32	64	64	32	0	128	0	16
Sindbis	16	4	4	64	64	0	32	0	0
	8	0	0	64	64	0	32	0	0
Semliki	16	64	32	64	16	0	64	0	16
	8	64	32	64	16	0	64	0	16
Chikungunya	16	16	32	32	16	0	64	0	32
	8	16	64	32	32	0	64	0	16
Mayaro	16	16	32	64	32	0	64	0	0
	8	0	0	0	0	0	64	0	0
Middleburg	16	16	16	32	32	0	0	0	0
	8	0	0	0	32	0	0	0	0
Normal brain	16	0	0	0	8	0	0	0	0
	8	0	0	0	0	0	0	0	0
Dengus 4 (Control)	16	0	0	0	0	0	0	0	0
	8	0	0	0	0	0	0	0	0
Diluent		0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0

* CF tests conducted by J. Casals, Rockefeller Foundation Virus Laboratories.
** Reciprocal of CF titers.

Record of Inoculations

Burro	VEE	EEE	WEE
GF 137	Col., 2/21/61	12/7/60	
GF 140	Col., 2/21/61	12/7/60	
GF 144	TC50, 9/21/59	1/13/60	11/7/61
	Col., 10/12/59		
GF 308		3/27/62	11/7/61
GF 325	Trin., 3/19/63	12/5/61	
GF 331	Trin., 3/19/63	3/27/62	
		5/10/62	

Results of these studies strongly suggest that EEE immune burros challenged with virulent Venezuelan equine encephalomyelitis (VEE) serum is broadly reactive CF antibodies. Quite possibly it will be necessary to supplement this immunization regimen by injecting other Group A arboviruses.

It would appear advantageous to produce monovalent as well as polyvalent typing sera in large animals provided that acceptable titers can be obtained.

An attempt was made to determine the effect of Sindbis virus on 4 WEE immune burros and on 4 sero-negative controls. No viremia was detected using tissue culture, and the antibody response was insignificant as measured by HAI, CF, NT tests.

Four burros having previous experience with Group A arboviruses and 4 sero-negative controls were challenged with Chikungunya virus in order to determine more definitely broad spectrum antibody response. Viremia titers were not measureable on tissue culture. Determination of antibody response is still in progress.

In another attempt to determine broad spectrum antibody response, 14 burros were challenged with WEE virus. Four of these burros had been previously exposed to EEE virus, 4 had prior experience with Sindbis virus, 2 had been vaccinated with attenuated WEE virus, and 4 had no prior experience with Group A arboviruses. Viremia detection by inoculation of day-old chicks and determination of antibody response by HAI, CF, and NT tests is in progress.

B. Avian infection with EEE.

Immune response in the domestic chicken to inoculation with viable EEE virus is being studied at present, as a cooperative venture by the U. of Md. and WRAIR. Various age groups of chickens are being inoculated over a 48-week period with EEE virus and are being bled at intervals to determine whether age at the time of injection influences the immune mechanism.

In the first group of chickens inoculated at one week of age, the peak antibody titer [HAI 1:2560] was reached at 2 to 3 weeks and leveled off after 6 weeks [HAI 1:160]. Detection of viremia in the first 3 groups of chickens was as follows:

	Viremia [range of NT index]			
Age of inoculation	Day 1	Day 2	Day 3	Day 4
2 wk	3.5 - 5.7	0 - 3.0	0	0
4 wk	4.0 - 6.3	0 - 2.5	0	0
8 wk	3.5 - 5.5	1.5 - 2.5	0	0

In the 8 week old chickens, viremia was detected in only 5 of 10 birds at day 2. It appears that the viremia level begins to drop off with an increase in age at the time of inoculation. This is a long-term project; the inoculation and testing schedule is not complete.

C. Guaroa and Cache Valley-like viruses.

1. Cache Valley-like virus. Vaccine was prepared from suckling mouse brains and used in combination with complete Freund adjuvant for the intraperitoneal inoculation of 2-month-old female mice over a 49-day schedule. At day 49, the abdominal region of the mice was considerably distended and ascitic fluid could be easily removed. It was found that repeated "taps" could be made at weekly intervals if the mice were injected again with vaccine and adjuvant.

   The ascitic fluid obtained was highly reactive in the CF and NT tests as immune material. CF antigen was prepared, but attempts to produce a hemagglutinin have been unsuccessful thus far.

2. Guaroa virus.

   These experiments were part of a contribution to the American Committee on Arthropod-borne Viruses, Subcommittee on Serological Reagents. Suckling mice for preparation of stock seed virus and adult mice for production of ascitic fluid and immune serum were inoculated according to the previously mentioned schedule. All material produced was tested for neutralizing antibodies. Stock seed virus was lyophilized and will be sent to the American Type Culture Collection and other interested workers upon request. Attempts were made to cultivate Guaroa virus on 33 different types of tissue culture; however, cytopathic effect was noted in only 6 lines of tissue culture (bovine embryonic kidney, WI 26, Flow 2051, Flow 2059, conjunctiva HEp II). CF and HA antigens were prepared. An attempt was made to induce and measure immunologic response in chickens and rabbits. Weanling and adult mice were found to be susceptible by intracerebral but not by intraperitoneal inoculation. Investigations are being continued on characterization of this virus in tissue culture and laboratory animals.

III. Other Studies.

A. Stored, frozen brains of horses and ponies from which EEE virus has been isolated over the past 6 years have been retested and lyophilized. This process will enable the laboratory to maintain a source of stock strains of EEE for future experimental work.

B. Vaccine studies. Laboratory tests have been completed on EEE vaccinated horse and pony sera from New Jersey and Virginia. Results indicate that even when using highly sensitive neutralization and hemagglutination-inhibition test systems, antibody response to vaccine is inconsistent.